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THE RELATIVE INFLUENCE OF MICROORGANISMS AND PLANT ENZYMS ON THE FERMENTATION OF CORN SILAGE

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PRELIMINARY DISCUSSION

HISTORICAL REVIEW

Ever since the fermentation of silage has been studied and discussed the question of the agent causing the fermentation has been in controversy. Some investigators have made the statement, based on evidence more or less incomplete, that microorganisms are solely responsible for the changes undergone by the ensiled forage. Other workers, who have based their conclusions on equally incomplete data, have held that in silage produced under proper conditions bacteria and yeasts do not figure to any appreciable extent, but that the plant cell itself is the cause of the chemical changes which take place in its constituents. Still other writers have sometimes taken sides on the subject without presenting any new data bearing on the problem.

Among the earliest workers on the chemistry and biology of silage formation were Burrill and Manns (3),[1] who found many species of bacteria in the silage, and stated that they were the cause of the chemical changes. Babcock and Russell (1) made silage in the presence of chloroform, ether, and benzene, obtaining in each case a change of color, some increase in acidity, and typical silage odor and flavor. These results and deductions made from other observations on the gases of the silo, the number of bacteria found, and on silage made from mature and immature corn led them to believe that bacteria were nonessential and that the cause of the fermentation was mainly the intramolecular changes which occur in protoplasm under anaerobic conditions when ordinary metabolic processes are suspended. Harding (8), working under their direction, found bacteria but no constant flora in silage. E. J. Russell (17) came to the conclusion that the primary and essential changes in silage fermentation were brought about

[1] Reference is made by number to "Literature cited," p. 378–380.

by the plant cell and its enzyms and that the changes caused by bacteria were secondary and nonessential. Esten and Mason (6) found such large numbers of bacteria and yeasts in silage that they considered them the only important factors concerned. Hunter and Bushnell (10) recently found large numbers of the *Bacterium bulgaricus* group in silage and considered their activities very important. All the work mentioned above has been done with silage made from the corn plant (*Zea mays*), which is the chief silage crop in this country.

Investigators in plant physiology have found evidence of the evolution of carbon dioxid and the formation of alcohol under aseptic conditions in the tissues of many plants, including maize. The distinction as to whether this is due to the action of enzyms within the cells or to respiratory activities of the cell protoplasm is not made; but similar respiratory changes appear to be common to the majority of plants, especially in their seeds. It has been suggested that the respiration of plants under anaerobic conditions is identical with alcoholic fermentation. In many cases some of the alcohol is further oxidized or otherwise changed, but the ratio of carbon dioxid to alcohol is often found to be comparable to that of ordinary alcoholic fermentation by the zymase of yeast. Of course, anaerobic conditions obtain in the silo after the first few hours. Doroféjew (4) found that the respiration of injured leaves was accelerated. This may have some significance in connection with the chopping of corn before it is ensiled. Zaleski and Reinhard (27) and others have noticed similar effects in wounded vegetable tissues. With various seeds placed under anaerobic conditions Godlewski and Polzeniusz (7), Stoklasa et al. (20, 21), Minenkoff (13), and a number of others have found that alcohol and carbon dioxid are produced. Similar results have been obtained by others with other plant tissues than the seeds. Although Mazé and Perrier (12) have questioned the results of Stoklasa, it is still possible that such respiratory activities play a part in the formation of silage.

Certain enzyms have been shown to be present in corn grain. A proteoclastic enzym has been found by Vines (24) and likewise by Scheunert and Grimmer (18), who found an amylase also present. Sigmund (23) found a lipase in both the resting and germinating seeds of maize. Price (15) demonstrated the presence of a peroxidase, a catalase, a protease, an invertase, and a glucosidase in cornstalks. White (26) found proteoclastic and amyloclastic enzyms in maize seeds, which retained their activity in seeds 20 years old.

STATEMENT OF PROBLEM

A sharp differentiation between the activities of enzyms and microorganisms in a given medium is practically impossible. Both are susceptible to injury and destruction by heat and are more or less similarly subject to the inhibitive effect of our common antiseptics. Moreover,

some of the principal manifestations of bacterial activity are identical with those of enzym action, as, for example, the evolution of carbon dioxid, production of alcohol, rise in temperature of medium, and hydrolysis of protein. Antiseptics, if used in high enough concentration to inhibit all bacterial growth, seem also to exercise a deleterious effect on the plant tissues and their enzyms. Aseptic conditions can be maintained for respiration experiments on small amounts of plant tissue, but it would be very difficult to produce silage under such conditions. The problem attacked was therefore to differentiate as accurately as possible between the results of the various activities of these two kinds of agents. A number of different experimental methods were employed in the effort to arrive at a distinction between them.

EXPERIMENTAL METHODS

Silage made in the laboratory in glass jars has been used in the greater part of this work. Experimental conditions can in this way be easily controlled, and comparisons are thus possible. The corn was chopped in a small silage cutter or was taken from the college farm silage cutter and packed as tightly as possible into cylindrical wide-mouth jars which were closed with rubber stoppers. Each was provided with an outlet tube for excess gases, which was closed with a pinchcock. Silage made in this manner is perfectly and normally preserved with characteristic appearance and aroma. Comparisons of chemical data between silage from the farm silos and from laboratory silos show no considerable difference. Of course, no two lots of silage are ever exactly alike chemically. The writer has previously made both corn silage and mixed silages in this manner (9, 11). Results obtained show no evidence that this laboratory silage is essentially different from silage made from similar material in an air-tight farm silo.

Some of the corn used in this work was grown to maturity in the greenhouse. Both greenhouse corn and field-grown corn were used, to prevent any possible abnormal results. The greenhouse corn was generally nearly as good in quality as the field-grown corn.

The analytical methods used are based upon the characteristic chemical changes which take place in silage fermentation. The formation of acids and alcohols, the evolution of carbon dioxid, the disappearance of simple sugars, and the degradation of protein, which are the principal chemical phenomena of the fermentation, have been measured by the methods described below. Results from methods based on these chemical changes show, as the conventional estimations of crude protein, fiber, ether extract, etc. do not, the nature and extent of fermentation and the character of the silage, as nearly as chemical analysis can show. In each case comparisons were made with similar figures obtained on samples of the green corn from which the silage was made. In each case the chemical determinations were made upon the juice expressed

from the silage in a Buchner press, under a pressure of 300 to 400 kgm. per square centimeter. This method of sampling facilitates the chemical examination, insures a well-mixed sample, and makes possible more comparable results.

The methods used are as follows:

TOTAL ACIDITY.—Ten c. c. of silage juice were diluted to about 500 c. c. with carbon dioxid free water, and titrated with decinormal barium hydroxid solution in the presence of phenolphthalein till a distinct pink appeared by reflected light against a white background.

VOLATILE ACIDITY.—One hundred c. c. of juice were distilled in a current of carbon dioxid free steam. To hasten the liberation of volatile acids and alcohols, 100 gm. of sodium chlorid were added to the juice. About 600 c. c. of distillate were titrated with baryta water in the presence of phenolphthalein.

ALCOHOLS (Distillation method).—The distillate from the volatile acid determination was neutralized with baryta water (solid phenolphthalein being added) and concentrated by repeated distillation with sodium chlorid (2). About 50 c. c. of alcohol solution were oxidized [1] in a pressure flask in a boiling water bath for 30 to 40 minutes, and the volatile acids then distilled off four or five times, with additions of carbon dioxid free water. The volatile acids were titrated and calculated as ethyl alcohol.

ALCOHOLS (Aeration method).—In this method (21) a current of air was drawn through the silage juice, which was saturated with ammonium sulphate, into concentrated sulphuric acid. The sulphuric acid-alcohol solution was then oxidized with the potassium dichromate solution, and distilled and calculated as in the previous method. Although other alcohols are formed in silage in small amounts, all were calculated as ethyl alcohol.

TOTAL SUGARS.—Fifty c. c. of juice were clarified with neutral lead acetate, the excess lead precipitated with anhydrous sodium carbonate, an aliquot allowed to stand 24 hours with hydrochloric acid, neutralized, and the total reducing sugars determined on an aliquot by either the Defren-O'Sullivan method or the slightly modified volumetric method of Schoorl (19). In any given series the same method was used to insure comparable results.

AMINO NITROGEN.—The amino nitrogen was determined on the diluted juice with the Van Slyke apparatus (22). This determination shows the relative degree of hydrolysis of protein if used on the same or similar material at successive periods.

AMMONIA NITROGEN.—A 50-c.c. sample of the juice was distilled with magnesium oxid, according to the official fertilizer method.

MOISTURE.—Samples of about 100 gm. of silage were dried to constant weight, in most cases in a vacuum oven at 60° C.

[1] The oxidizing solution used was made up in the following proportions: 10 gm. of potassium dichromate ($K_2Cr_2O_7$), 20 gm. of sulphuric acid (H_2SO_4), 70 gm. of water.

SILAGE MADE UNDER ANTISEPTIC CONDITIONS

Corn silage made in the presence of ether and chloroform, as done by Babcock and Russell (1), is, of course, well preserved and evolves, after the antiseptic has been allowed to evaporate, an odor quite aromatic and characteristic of silage. The exact amount of antiseptic which should be added to inhibit bacterial growth without seriously impairing enzymic action is, however, very difficult to estimate. Moreover, Wagner (25) has found that certain bacteria may flourish in the presence of benzene, phloroglucinol, phenol, and phenolic derivatives. Some experimental silage was made, however, in the presence of chloroform, toluene, and cresol. Analytical data on these are shown in Table I.

TABLE I.—*Analytical data on antiseptic silage*

[Calculated on basis of 100 gm. of dry silage.]

Kind of silage.	Total acidity (*N*/10).	Alcohol.	NH_3–N.
	C. c.	*Gm.*	*Gm.*
Typical green corn	34	0	0.052
Toluene silage (2 per cent)	31	Trace.	.057
Chloroform silage (5 per cent)	250	.....	.176
Cresol silage (0.5 per cent)	255	0.15	.199
Normal silage	675	.81	.256

The amount of toluene added seems to have been enough to stop practically all change. No bacterial growth was obtained from the chloroform silage. It seems likely that the jar of cresol silage contained a limited number of active organisms, as two forms were isolated from this silage, one of them an acid former, and no evidence of spore formation could be obtained with either of these organisms. Some of the results of the work with antiseptics have been introduced here to show the apparent impossibility of obtaining conclusive results, at least with silage, by using such methods alone. These data, however, may be of some value when compared to other data shown on the following pages. Further experiments with varying amounts of antiseptics were not attempted, as it seemed likely that other methods would give more conclusive results.

OTHER EXPERIMENTAL SILAGE

An effort was made to learn the effect of bacteria and yeasts alone, by heating jars of chopped corn to destroy the enzyms, followed by inoculation with an infusion of normal silage, which should carry the normal mixed flora of silage. After inoculation the jars were in each case incubated at 28° to 30° C., with a control jar of normal silage made from the same sample of corn. The analytical data, which are interesting but not conclusive, are shown in Table II.

TABLE II.—*Silage heated and inoculated*

[Data on 100 c. c. of juice]

Kind of silage.	Total acidity (*N*/10).	Volatile acidity (*N*/10).	Alcohol.	NH_3-N
	C. c.	*C. c.*	*Gm.*	*Gm.*
Green corn	23	1.8	0	0.044
Normal silage (control)	375	118.0	.265	.....
Corn heated to 80° and inoculated	274	123.6	.343	.135
Green corn	43	.....	0	.111
Normal silage	318	109.6	.150	.214
Corn heated to 90° and inoculated	198	99.1	.244	.068
Green corn	19.5	1.2	0	.024
Normal silage	342.5	73.1	.207	.114
Corn heated to 85° and inoculated	301.5	58.4	.188	.035

The prominent part which may be played by yeasts in the fermentation of silage under certain conditions was demonstrated by adding to a jar of silage sufficient tartaric acid in solution to make 2 per cent of the weight of the silage. An acid mixture of this strength practically inhibits bacterial action and favors the development of yeasts. A comparison of the acidity of the silage with the amount of tartaric acid added showed that evidently no other acid had been formed, except some volatile acid, which might possibly have come from the oxidation of alcohol. The quantity of alcohol found in 100 c. c. of this silage juice was 1.746 gm., expressed as ethyl alcohol, while normal silage juice contains only from 0.20 to 0.45 gm.

RATE OF CHEMICAL CHANGES IN SILAGE FERMENTATION

A more conclusive method of differentiating between the activities of enzyms and of microorganisms was suggested by a paper by Rahn (16), who discussed the usefulness of curves in the interpretation of microbial and biochemical processes. It is shown by Rahn that the curve which is obtained when the formation of products of fermentation or other biochemical process is plotted, taking as abscissæ the total time elapsed and as ordinates the total amounts of compounds produced, is in many cases indicative of the nature or cause of the change. If the change is caused by enzymic action and is, therefore, purely chemical, the active mass of the agent causing the change does not increase as the reaction progresses; and the decreasing concentration of the substance acted upon and the accumulation of end products tend to decrease the rate of change. Thus, the curve becomes convex toward the Y axis. The mass of enzym does not increase unless there are living cells present to elaborate more enzym. However, if organisms are present and are active, they multiply until the exhaustion of nutrients or the accumulation of end products retards and finally stops their increase. Until this time the

rate of change increases with the increase in number of organisms, and the resulting curve is convex toward the X axis. Then there is a point of inflection, after which the curve becomes similar to the enzymic curve. An example of a curve of this type is shown in figure 1, a typical "fermentation curve." There is always a point of inflection, or change in direction of curvature in a bacterial curve, provided the data begin before the number of organisms has reached its maximum. These two kinds of curves are discussed at some length by Rahn in the article cited (16).

In natural or mixed fermentations, such as the formation of silage, it is possible that different processes taking place at the same time will destroy the natural form of the curve. Or if both chemical and biological factors are present and producing the same substance, e. g., alcohol, the nature of the curve might be variable, depending upon the relative "active mass" of the two agents. In the case of so large an inoculation that the bacteria do not multiply materially, a bacterial curve might possibly resemble the curve of an enzymatic process. However, the chemical composition of the material and the extent of the fermentation depend upon many variable factors, such as soil and meteorological conditions, the method and rate of filling the silo, and opportunity for inoculation. The silage resulting is thus a variable product, depending on these and other factors. Therefore it seems very unlikely that any considerable error in the interpretation of curves drawn from silage data would persist through a number of entirely separate experiments. This is evident from the data given in the following pages.

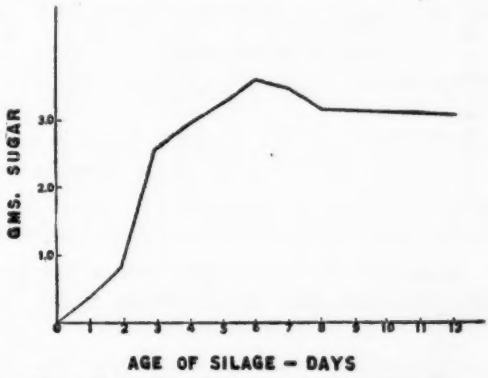

FIG. 1.—Curve showing the disappearance of sugars in series 1.

The analytical methods outlined above were used to obtain data on the chemical changes occurring during the early period of silage fermentation, in order that curves such as just described might be plotted. The data obtained by Neidig (14) on this part of the fermentation process indicate the impossibility of obtaining regular curves and strictly comparable results by taking samples from a farm silo, owing to the variability in composition of the silage in different parts of the silo, and the necessity of the perfect exclusion of air throughout the process. Therefore, silage was made in the laboratory in small jars as mentioned above. The chopped corn was very thoroughly mixed, and after a sample had been taken for the initial analysis, it was packed tightly into jars.

The jars were kept under the same conditions, generally in an incubator at 28° to 32° C., following the average rise of temperature in the silo. One jar was opened each day and the juice pressed out and analyzed. This method should give as nearly correct results on the rate of change as it is possible to obtain, it being granted that silage formation in small jars is perfectly normal. The changes which were found were very similar to the changes observed by Neidig in the farm silos, except that the uniformity of the samples gave much more uniform and regular curves.

TABLE III.—*Series 1: Formation of acid and alcohol, disappearance of sugars, and increase in animo nitrogen in silage*

[All data calculated to sample of 100 c. c. of silage juice.]

Age of silage.	Total acidity N/10 sol.	Alcohol.	NH_3—N.	Total sugars.	Total sugars which disappeared.
Days.	*C. c.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
0	27.5	0.002	0.069	5.299	0.000
1	47.5	.144	.083	4.934	.365
2	99.7	.065	.089	4.526	.773
3	166.2	.067	.098	2.774	2.525
4	190.0	.082	.089	2.323	2.976
5	233.7	.090	.106	2.078	3.221
6	253.6	.129	.108	1.742	3.557
7	278.3	.095	.109	1.867	3.432
8	226.1	.082	.109	2.203	3.096
12	228.0	.114	.109	2.270	3.029

A series of nine jars of silage (series 1), using corn grown to maturity in the greenhouse, gave very interesting results. The corn was mixed and ensiled as described above. The determinations on the green corn and on each day's sample of silage juice were made, as usual, in a strictly comparable manner. The analytical data are given in Table III.

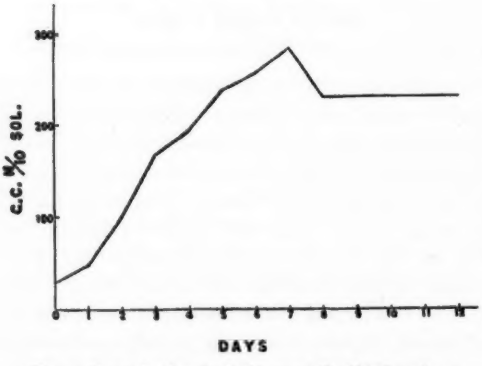

FIG. 2.—Curve showing the development of acidity in series 1.

The curves plotted from the data in Table III are shown in figures 1 to 4. The curves showing the disappearance of sugars and formation of acids are similar in shape and are typical of bacterial fermentation. The amino-nitrogen curve, which shows the rate of hydrolysis of protein, and the alcohol curve both show the abrupt rise at the beginning which characterizes the enzymic type of

curve. However, between the second and third days during the same period in which there appears a marked increase in bacterial activity in the sugar and acid curves, there is also a second rise in the NH_2–N curve, which is probable evidence of some proteoclastic action by bacteria.

A similar series of determinations (series 2) made two months later shows a somewhat different set of curves. Field-grown corn at the proper stage of maturity was chopped in the silage cutter, inoculated with material carrying the usual flora of the farm silage cutter, and ensiled as before. In this series total and volatile acid, alcohol, sugars, amino nitrogen, and ammonia nitrogen were determined. The analytical data are shown in Table IV.

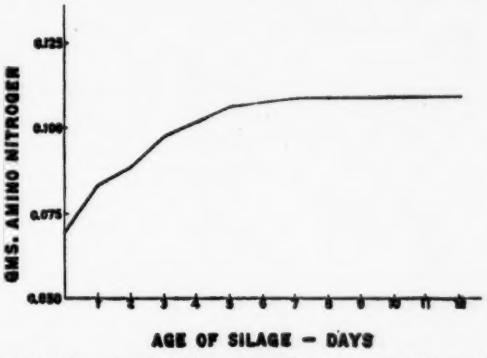

FIG. 3.—Curve showing the increase in amino nitrogen (and the rate of hydrolysis of protein) in series 1.

TABLE IV.—*Series 2: Formation of total and volatile acid and alcohol, and the disappearance of sugars, amino nitrogen, and ammonia nitrogen in silage*

[Data on 100 c. c. of silage juice.]

Age of silage.	Total acidity (*N*/10).	Volatile acidity (*N*/10).	Alcohol.	NH_2–N.	NH_3–N.	Total sugars.	Disappearance of sugars.
Days.	*C. c.*	*C. c.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
0	17.3	1.4	0.000	0.021	0.006	3.139	0.000
1	97.0	42.1	.188	.053	.008	1.622	1.517
2	160.3	56.9	.210	.060	.010	1.008	2.131
3	205.4	65.2	.197	.066	.010	.648	2.491
4	235.2	68.4	.262	.095	.026	.235	2.904
5	250.6	68.6	.307	.098	.018	.158	2.981
7	254.4	70.6	.238	.097	.019	.019	3.120
9	263.0	71.0	.403	.104	.021	.019	.....
12	266.9	78.9	.256	.084	.019	.120	.....
15	274.6	75.8	.347	.112	.025	.161	.....
21	291.8	84.0	.354	.128	.027	.....	.....
30	296.6	79.5	.337	.149	.028	.....	.....

The form of the acid curves of this series (fig. 5), when compared to the usual form of the acid curves, suggests the possibility of so large an inoculation with acid-forming bacteria that the maximum in numbers was reached during the first 24 hours. The curve showing the disappearance of sugars (fig. 6) has the same form. The curve showing the formation of alcohol (fig. 4) is of the enzymic form, like the corresponding curve in series 1. Irregularities in these two alcohol curves

suggest the possibility that each is the resultant of the formation of alcohol by one or more agents and its simultaneous oxidation by other agents. Or there might possibly be some variation between individual samples in the series. The amino-nitrogen curve (fig. 7) shows an evident enzymatic protein hydrolysis during the first three days. Between the third and fourth days, however, an abrupt rise takes place, which bears out the assumption made from series 1, viz, that bacteria figure in the hydrolysis of protein after the first two or three days. It is noteworthy that a similar rise takes place during the same period in the sugar, alcohol, and ammonia-nitrogen curves, indicating a general increase in the activity of microorganisms at that time. As previously mentioned, just such a simultaneous rise was noticed in three of the curves in series 1 between the second and third days. This observation lends strength to the evidence in favor of enzymic action in these cases during the first two days.

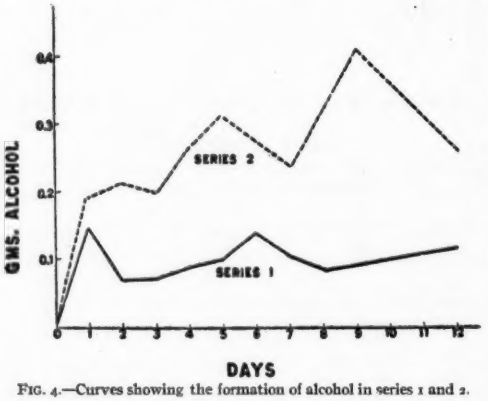

FIG. 4.—Curves showing the formation of alcohol in series 1 and 2.

Corn grown in the greenhouse was used for series 3. This corn made an excellent growth and was practically as good in quality as field-grown corn. The corn was chopped in the laboratory and ensiled as before, but with little opportunity for inoculation. Analytical data on this series are shown in Table V. The first jar of silage was opened when only 12 hours old.

TABLE V.—*Series 3: Formation of acids and alcohol and disappearance of sugars*

[Data on 100 c. c. of silage juice.]

Age of silage.	Total acidity (*N*/10).	Volatile acidity (*N*/10).	Alcohol.	Total sugars.	Disappearance of sugars.
Days.	*C. c.*	*C. c.*	*Gm.*	*Gm.*	*Gm.*
0	14. 0	2. 7	0. 001	3. 850	0
½	25. 0	2. 8	.079	4. 292	− .442
1	36. 5	2. 9	.123	4. 060	− .210
2	39. 0	3. 0	.272	3. 428	+ .422
4	41. 5	3. 1	.312	2. 236	1. 614
7	63. 5	5. 0	.414	1. 660	2. 190

The increase in the amount of sugars during the first day is interesting, and is very probably due to the presence in the corn grain of amylase,

which in this case produced sugar from higher carbohydrates faster than the sugar was used. The development of acids is remarkably small, due perhaps to the slight opportunity for inoculation with acid-forming organisms. It is unfortunate that a larger number of jars was not filled in this experiment so that the later progress of acid formation could have been followed. Since there is no possibility in this series that the formation of acetic acid complicates the question of alcohol formation, some interesting observations are possible. The alcohol curve (fig. 8) after beginning like an enzymic curve takes another abrupt rise between the first and second days, probably when the yeasts become more active. The concomitant production of carbon dioxid in the silage of this series was also measured. (The data are given on page 374.) The carbon-dioxid curve (fig. 13) shows the same trend throughout as the alcohol curve, including the rise just mentioned between the first and second days. The carbon dioxid : alcohol ratios show a general increase as the fermentation progresses, as follows:

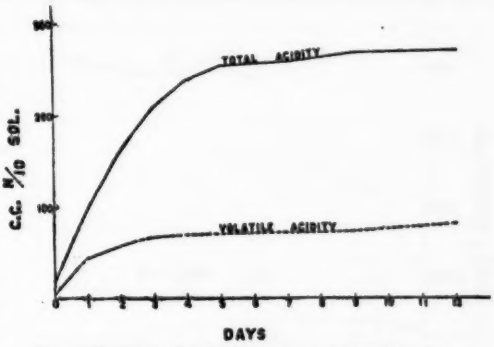

FIG. 5.—Curves showing the development of acidity in series 2.

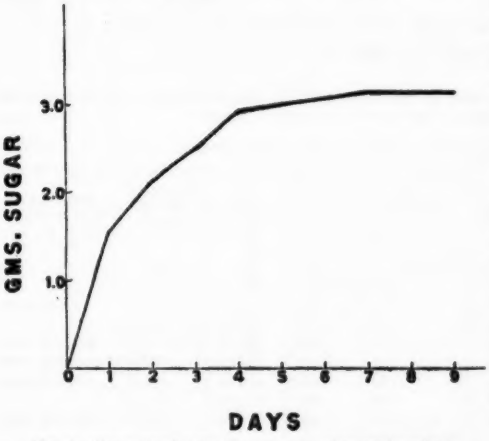

FIG. 6.—Curve showing the disappearance of sugars in series 2.

Age of silage (days)	½	1	2	4	7
Ratio	1:0.51	1:0.61	1:0.86	1:0.79	1:0.97

This increase might be either because the yeasts are taking an increasingly greater part in the fermentation and the ratio therefore approaches the ratio of the ordinary alcoholic fermentation, or because carbon dioxid from other reactions is included in the amount evolved during the first few days. Of course, the above is the result of but a single experiment on this point.

In the next series (series 4) the corn used was from the same greenhouse plot as the preceding. The use of the same tools, etc., a week later, gave opportunity for a much larger inoculation with acid-forming bacteria. This is evidenced by the data shown below. In this series bacteriological counts of some of the samples were made.[1] The technic used was as follows: The sample was ground in a sterile mortar for 15 minutes, 50 gm. weighed out, placed in a liter flask with 500 c. c. of sterile water and shaken 100 times. One c. c. was plated in various dilutions in a yeast-extract agar and incubated for 48 hours at 37° C. Data on this series are given in Table VI.

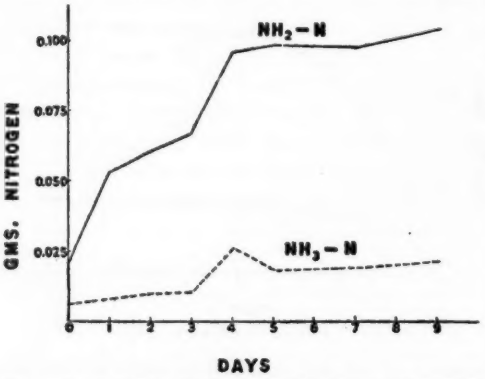

FIG. 7.—Curves showing the increase in amino nitrogen and ammonia nitrogen in series 2.

TABLE VI.—*Series 4: Formation of acids and alcohol, disappearance of sugars, amino nitrogen, and bacterial counts in silage*

[Data all on 100 c. c. of juice, except bacterial counts.]

Age of silage.	Total acidity (*N*/10).	Volatile acidity (*N*/10).	Alcohol.	NH_2–N.	Total sugars.	Disappearance of sugars.	Bacteria per gram of silage.
Days.	*C. c.*	*C. c.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	
0	15. 5	2. 7	0. 001	0. 028	6. 662	0	.....
½	39. 0	3. 5	. 109	. 058	7. 036	− . 374	9, 900, 000
1	55. 5	4. 6	. 176	. 076	7. 594	− . 932	.....
2	121. 0	7. 1	. 192	. 108	5. 030	(+)1. 632	106, 500, 000
3	156. 0	18. 9	. 193	. 120	3. 764	2. 898	119, 000, 000
4	148. 5	24. 5	. 182	. 116	4. 636	2. 026	38, 800, 000
6	223. 5	47. 8	. 197	. 101	2. 324	4. 338	.....
9	254. 0	58. 2	. 268	. 107	2. 190	4. 472	106, 000, 000

It should be noted that the preliminary increase in sugars occurs as it did in series 3. The comparatively small bacterial count in the 4-day-old silage is reflected in the total acid, amino nitrogen, and sugar columns. The alcohol curve (fig. 8) shows the same enzymic form as before. The acid curves (fig. 9) show the usual bacterial form. The amino-nitrogen curve (fig. 10) is of the enzymic form as before. The curves showing the disappearance of sugar in series 3 and 4, though not reproduced here, approach the bacterial form.

[1] Acknowledgment is gratefully made to Dr. R. E. Buchanan for the use of laboratory facilities and media for making these counts.

A final lot of silage (series 5) was made from corn grown in the field during the present year. This corn lay in the field or on wagons 15 to 20 hours after cutting. It was taken from the farm silage cutter, mixed, and ensiled as before. The data are given in Table VII.

TABLE VII.—*Series 5: Formation of acids and alcohol in silage*

[Data on 100 c. c. of juice]

Age of silage.	Total acidity (*N*/10).	Volatile acidity (*N*/10).	Alcohol.
Days.	*C. c.*	*C. c.*	*Gm.*
0	45. 0	6. 0	0. 022
½	47. 5	6. 5	. 190
1	123. 5	7. 5	. 269
2	218. 5	36. 8	. 342
3	263. 0	45. 3	. 515
4	312. 5	65. 2	. 465
7	362. 5	96. 8	. 633

The initial acid and alcohol content are rather high, as the corn stood so long after cutting. The acid curves (fig. 11) show the usual bacterial form and the alcohol curve (fig. 12) the usual enzymic form, with the later rise presumably due to yeasts.

The data obtained from these five series of experimental silage will be discussed below.

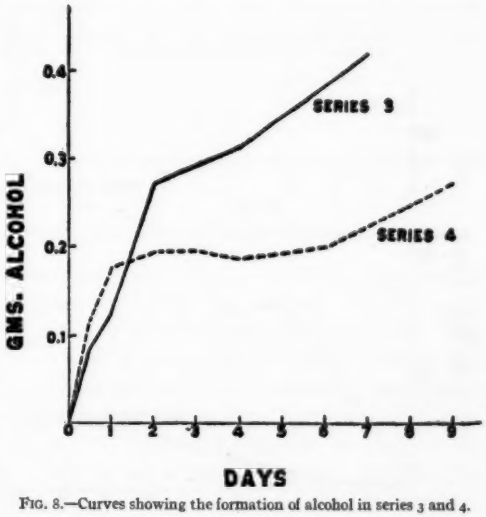

FIG. 8.—Curves showing the formation of alcohol in series 3 and 4.

EVOLUTION OF CARBON DIOXID

The amount of carbon dioxid evolved by silage, a constant and characteristic phenomenon of the process, was measured by absorption in caustic potash solution. The silage was packed into cylindrical specimen jars with wide mouths which were fitted with specially made rubber stoppers. A ⅛-inch galvanized-iron pipe was led to the bottom of each jar and the corn was tightly packed around it. This pipe was closed at the top with a rubber tube and a pinchcock. An outlet tube at the top of each jar was connected to an absorption train. The gas was forced through the train by its own pressure, which was always greatest during

the first day, gradually decreasing thereafter. At the end of each period the iron inlet tube of one of the jars was connected to a soda-lime tube and a current of air was drawn through the absorption train for 30 minutes to remove the carbon dioxid remaining in the jar. The silage could then be removed for analysis. The data from the three experiments are given in Table VIII.

TABLE VIII.—*Evolution of carbon dioxid in silage*

[All data calculated to sample of 100 gm. of silage.]

Age of silage.	Evolution of carbon dioxid.[a]		
	Experiment 1 (Gm. CO_2).	Experiment 2 (Gm. CO_2).	Experiment 3 (Gm. CO_2).
Days.			
½	.....	.....	0.115
1	0.288	0.228	.148
2	(b)	.404	.226
3	.341	.490	.....
4	(b)	(b)	.292
5	(b)	(b)	.....
6	.....	.508	.....
7	.....	.....	.315

[a] These data are from series 3, p. 370. [b] Determination lost.

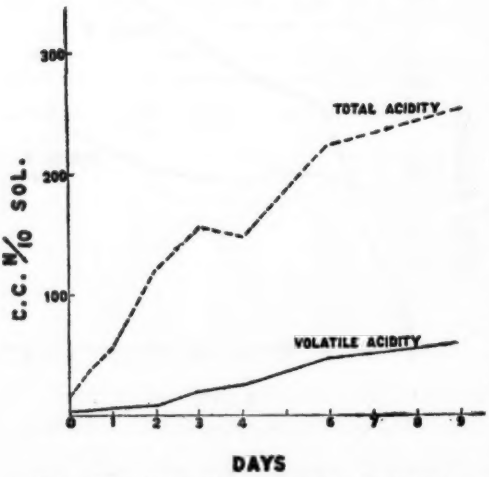

FIG. 9.—Curves showing the development of acidity in series 4

The curves plotted from these data are shown in figure 13. All are of the enzymic form, and check with the observations of the writer on all the silage he has made, viz, that the evolution of gas is always greatest during the first day or two, and nearly ceases after about four days. In most cases the rate of evolution is evidently kept up after the first day or two by contributions from bacteria and yeasts. In experiment 3 the curve shows a change of direction and a distinct rise during the second day, coincident, as remarked above, with a similar rise in the alcohol curve in the same experiment (cf. series 3).

RISE IN TEMPERATURE

Another characteristic phenomenon of silage fermentation, but one very much misunderstood in the early days of silage making, is the rise in temperature of the silage. Temperatures as high as 130° F. have been observed in the silo at or near the surface of the silage. This excessive heating is due to activity of microorganisms greatly accelerated by the presence of atmospheric oxygen, and occurs whenever silage is uncovered and left exposed to the air for a time. The temperature deep in the silo, however, protected from the air and sufficiently removed from the conduction of heat from the surface of the silage, is rarely higher than 80° to 90° F. It is rather unsatisfactory to attempt to obtain curves characteristic of bacterial or enzymic fermentation from the rise in temperature of the medium, on account of the number of somewhat extraneous factors involved. The outside temperature is always a factor, and the rise in temperature of the silage might easily affect the rate of chemical reactions or of bacterial growth, thus increasing the rate of temperature rise and perhaps changing the nature of the curve. Data which have been obtained from the farm silos[1] and from very carefully insulated laboratory silos suggest that the greater part of the heat developed is due to microbial action. It is considered unnecessary to reproduce these data here, as similar data have been published (14). However, one table is subjoined (Table IX) showing the rise in temperature at the surface of the silage in one of the farm silos. An iron pipe was forced down into the silage for about 4 feet, and a thermometer, immersed in a test tube full of water, lowered into the pipe so that the bulb was 2 feet below the surface of the silage. The top of the pipe was closed except when the thermometer was pulled up for reading. These data when plotted give a smooth and typical bacterial fermentation curve. Of course, this does not exclude the possibility of some heat production by enzym action.

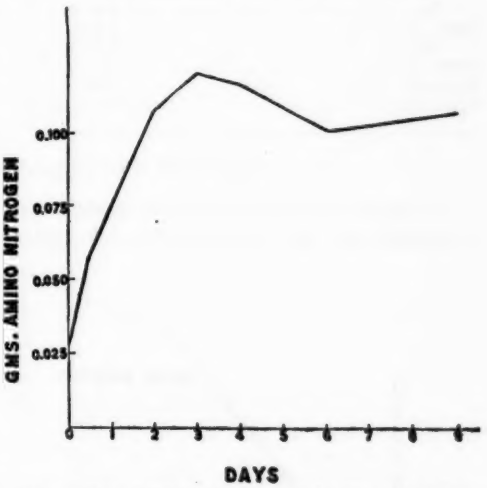

FIG. 10.—Curve showing the increase in amino nitrogen in series 4

[1] With the cooperation of the Agricultural Engineering Section of the Iowa Station.

TABLE IX.—*Rate of heating at surface of silage in brick silo*

Date.	Time.	Age of silage.	Temperature.	Outside temperature.
		Days.	°F.	°F.
Sept. 19	4 p. m.	0	71.2	61
Do	12 mid.		75.4	
Sept. 20	9 a. m.		82.0	67
Do	12 m.		85.6	
Do	6 p. m.	1	100.8	
Sept. 21	9 a. m.		113.4	58
Do	12 m.		115.3	
Do	4 p. m.	2	117.6	
Sept. 22	9 a. m.		122.2	53
Do	6 p. m.	3	123.2	
Sept. 23	12 m.	4	125.6	52
Sept. 24	12 m.	5	127.2	61
Sept. 25	12 m.	6	128.8	62

DISCUSSION AND CONCLUSIONS

It appears that neither microorganisms nor plant enzyms are alone responsible for the changes which take place in corn silage fermentation. The curves in these pages show that acid production is mainly if not entirely a phenomenon of bacterial activity in the silage. The results from the other experimental silage described above also suggest that the greater part of the acid is produced by microorganisms.

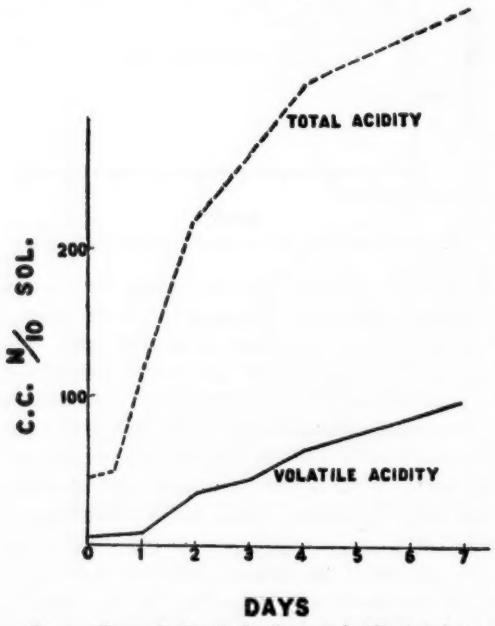

FIG. 11.—Curves showing the development of acidity in series 5

The curves showing the disappearance of sugars are, like the acid curves, generally of the bacterial fermentation type. Although some of the sugar is undoubtedly changed by enzym action, the greater amount seems to be metabolized by bacteria and yeasts.

The formation of alcohol, however, is evidently a phenomenon primarily of the respiratory or enzymatic activity of the plant cells. This is

suggested by investigators in plant physiology, who have often found zymase in plants, and is corroborated by the nature of the alcohol curves shown. The curves suggest a later production of alcohol by yeasts, and results from the other experimental silage support this idea. As stated above, Esten and Mason (6) found large numbers of yeasts in corn silage. Both factors, therefore, probably have a share in the alcohol production.

A similar statement holds good for the hydrolysis of protein as indicated by the amino-nitrogen content of the silage. Proteoclastic enzyms are present in corn grain, and the curves show evidence of their activity. Both the later rise in the amino-nitrogen curves noted above and the results from silage in which the enzyms were destroyed show some proteoclastic activity by microorganisms also. It is noteworthy that E. J. Russell (17) found end products of protein hydrolysis in corn silage made in the presence of toluene, which hydrolysis he ascribed to enzym action.

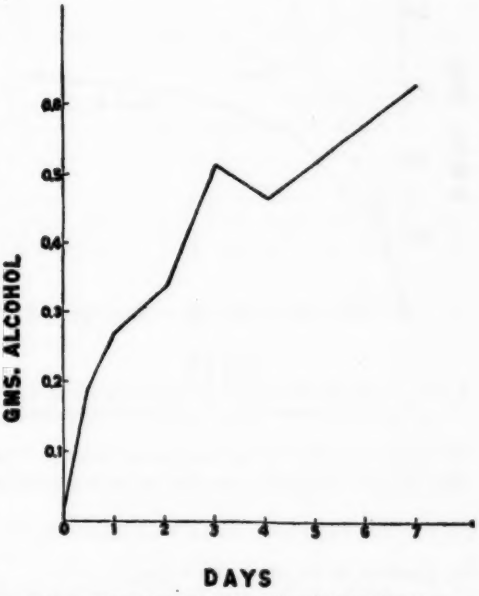

FIG. 12.—Curve showing the formation of alcohol in series 5.

The evolution of carbon dioxid must be due largely to enzym action. The curves shown all agree on that point, even when the first period was only 12 hours. Evidence that yeasts produce a part of the carbon dioxid after the first day has also been pointed out.

The rise in temperature of the silage is not great except at the surface, where the material is in contact with air. Microorganisms seem to be responsible for most of the heating, but the partial influence of enzyms is not excluded.

SUMMARY

The question of the respective causal relationship of microorganisms and plant cell enzyms to the fermentation of corn silage has long been in controversy. It is difficult to differentiate between the activities of these two kinds of agents. Work with antiseptics both by earlier investigators and by the writer is not conclusive. Experimental silage, other

than antiseptic silage, has been made, with results of some value; but the most conclusive evidence is obtained by the determination of the rate of change in various phenomena of the fermentation under normal conditions. Curves plotted from these data show that bacteria are mainly responsible for acid production and the concomitant disappearance of sugars. Alcohol is formed first by plant enzyms and later by yeasts. Protein is hydrolyzed first by enzyms and later by microorganisms. Carbon dioxid evolution seems to be very largely due to respiratory or enzymic activities, but yeasts probably have a share in its production after the first day or two. Microorganisms are probably largely responsible for the heating of the silage. Both kinds of factors are always present during silage fermentation and the process is due to the activities of both in the absence of air.

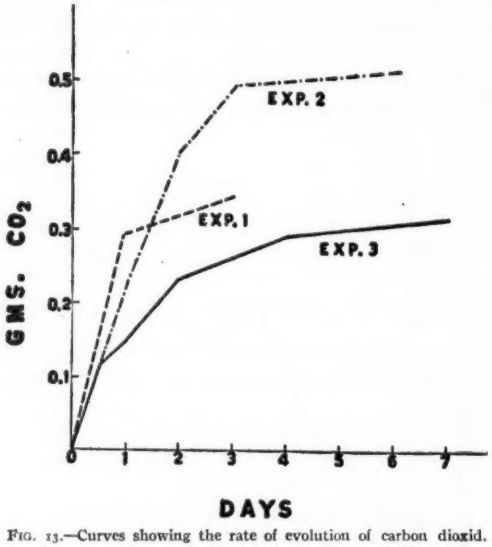

FIG. 13.—Curves showing the rate of evolution of carbon dioxid. (Curves 2 and 3 are coincident during the first one-half day.)

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LITTLE-LEAF OF THE VINE

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OCCURRENCE OF THE DISEASE

A disease of the vine (*Vitis* spp.) known variously as "little-leaf," "curly-leaf," and "yellow-leaf" first attracted attention in California about the beginning of the century. Some growers claim to have noticed it earlier, but no printed reference has been found that points clearly to this disease before 1900. It seems probable that it was present earlier, but most of the vines in many districts where it is prevalent have been planted since that date. This may account for the apparent spread and increase of the disease during the last 10 or 15 years.

At present, many vines are attacked in various regions, from the borders of Sacramento and San Joaquin Counties to the southerly end of the San Joaquin Valley. No indubitable cases have been noted in the Sacramento Valley, south of Kern County, or in any part of the coast region. The total area attacked is difficult to estimate, but it is large. In some localities, only occasional vines or small spots are affected; in others, most of the vines show more or less intense symptoms.

LOSSES

The loss in crop is also large and equally difficult to estimate. In some regions the affected spots are fairly definite, the vines badly injured, and the crop almost a total failure. These regions, however, represent the smallest part of the loss. Where the disease attacks the vines with great severity, if the affected spots are large, the whole vineyard is usually abandoned. It is only where the diseased spots are small that they are allowed to remain. The greatest loss occurs in the vineyards where the disease appears in a milder form and is distributed over wide areas. In such cases the crop may be diminished one-half, one-third, or less. The total loss of crop for California from little-leaf is considered by some observers to be greater than that due to any other vine disease. This is probably an exaggeration; but undoubtedly it takes rank with mildew, vine hoppers, and phylloxera as one of the most destructive.

GENERAL CHARACTER OF THE DISEASE

A vine, when very slightly affected, shows nothing but a few lightish areas or broad stripes on some of the larger leaves near the middle of the cane. These are not very noticeable and appear to be due to a slight deficiency of chlorophyll in the parts affected. On such vines the crop

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may be nearly normal, but there are usually many abortive berries ("shot grapes"). Vines badly affected show small yellowish leaves, short joints, upright canes, and abnormally numerous laterals (Pl. 89, *A*). The blossoms on such vines may all drop without setting, or a few normal berries may form on some of the bunches. Such vines seldom yield more than half a normal crop. Very badly affected vines show the same symptoms in an intensified degree, and toward the end of summer many of the leaves turn brown in whole or in spots. These vines bear nothing, and usually die after a year or two.

The appearance of a badly affected vine is very distinct, and the symptoms are unmistakable. They suggest very strongly a specific disease; but no bacterial, fungus, or animal parasite has been found in connection with them.

RELATION TO ENVIRONMENT

DISTRIBUTION IN THE VINEYARD

The distribution of the diseased vines is of two general types, which may be called the "diffusive" and the "intensive."

The diffusive type occurs usually in the lightest sandy soils. Here every vine in the vineyard may show symptoms. The disease in this case is seldom or never severe enough to kill the vines, but may seriously diminish the crop. It varies with the year and the variety. Some varieties may be almost immune, and in some years very few vines show the effects of the disease.

The intensive type occurs usually in sandy loams. In some cases of this type, a few badly diseased vines, even a single one, may be found completely surrounded by apparently perfectly healthy vines. Generally the diseased vines occur in irregular, ill-defined spots. Most of the badly diseased vines are found more or less in groups, but some of them are found among the surrounding healthy vines and sometimes a healthy vine will be found surrounded by a group of badly diseased. Healthy vines are seldom found near the middle of a large group of badly diseased vines.

Plate 92, *B*, and text figure 1 illustrate the intensive type of distribution. Figure 1, *A*, represents a vineyard where the attack is moderate and which is still producing paying crops. There is no evidence that the disease has killed any vines, as the number of vacancies (2.1 per cent) is quite normal and they occur among healthy and diseased vines with about equal frequency. Only 17.9 per cent of the vines are so badly diseased that they produce practically no crop; 26.6 per cent are noticeably affected and produce, on the average, about one-half of a normal crop. The remainder, 53.4 per cent, are apparently healthy. Such a vineyard would produce probably about two-thirds of a normal crop.

Figure 1, *B*, represents a very bad case of the disease. The vacancies represent 20.3 per cent of the area, and they are most frequent among

the worst vines. It is evident that the disease has killed many. Badly diseased vines represent 43.7 per cent, slightly diseased 13.7 per cent, and healthy vines 22.3 per cent. Such a vineyard would yield only about one-fourth of a normal crop.

CHARACTER OF THE SOIL

Typical or serious cases of little-leaf have been noted only in sandy or sandy-loam soils. If one passes through an infected region, the disappearance of the disease with a change from sandy to clay soil is very noticeable. The few cases in which the symptoms of little-leaf have been noted on vines growing in heavy soils do not seem to be typical and can probably be attributed to the soil of old sheep camps, which affects both vines and fruit trees in a similar way.

Perfectly healthy vines are found growing in sandy soil which on superficial inspection seems to be identical with adjoining soil where the vines are diseased. The soils in cases of this kind were examined in the attempt to determine whether any chemical or physical differences could be found which might account for the disease.

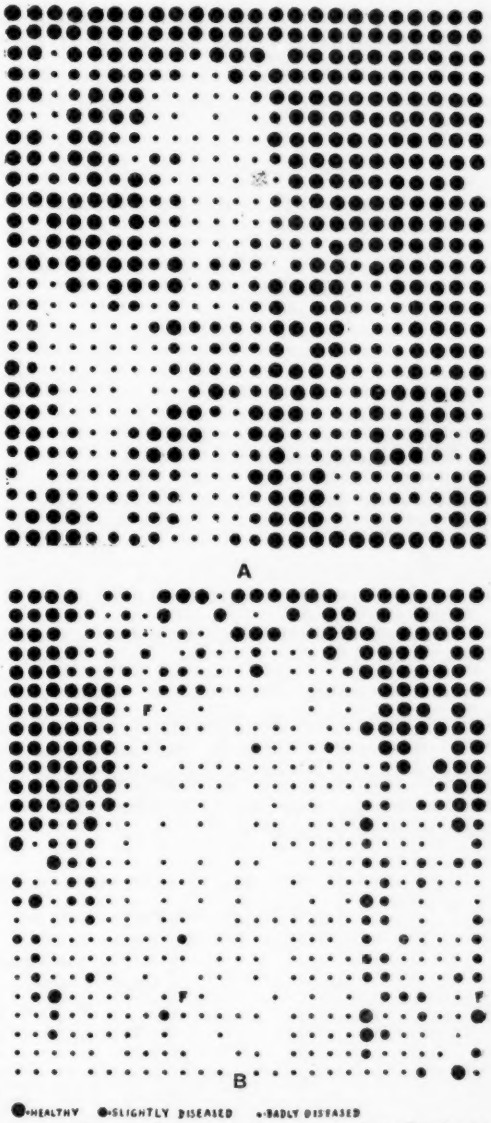


FIG. 1.—Diagrams of the distribution of grapevines affected with little-leaf: *A*, Moderately affected vineyard; *B*, a very badly affected vineyard.

Sample I was taken from a soil on which all the vines were healthy. Samples II and III were obtained from samples of soil taken from the same vineyard about 300 yards from Sample I. Sample II was taken from between healthy vines, and Sample III from between diseased vines.

The chemical analyses of these three soils show such similarity of composition that they throw no light on the cause of the disease (Table I).

TABLE I.—*Chemical composition of vineyard soils (W)*

[Analysis made by the Division of Soil Bacteriology, University of California.]

Constituent.	Sample I (soil bearing healthy vines).		Sample II (soil bearing healthy vines).		Sample III (soil bearing diseased vines).	
	1 ft.	2–4 ft.	1 ft.	2–4 ft.	1 ft.	2–4 ft.
Insoluble matter	72.98	70.75	75.87	075.21	074.26	74.77
Soluble silica	10.02	11.40	8.03	8.10	8.86	9.18
Potash (K_2O)	.80	.83	.47	.51	.59	.59
Soda (Na_2O)	.20	.35	.26	.23	.23	.24
Lime (CaO)	1.25	1.22	1.16	1.21	1.11	1.11
Magnesia (MgO)	1.11	1.15	.88	.91	.88	.93
Brown oxid of manganese (Mn_3O_4)	.11	.07	.08	.06	.05	.05
Peroxid of iron (Fe_2O_3)	4.38	4.88	5.94	6.15	5.50	5.60
Alumina (Al_2O_3)	5.96	6.97	5.29	5.85	5.55	5.73
Phosphoric acid (P_2O_5)	.19	.14	.18	.13	.12	.10
Sulphuric acid (So_3)	.05	.21	.05	.04	.05	.05
Water and organic matter	3.20	2.47	2.16	1.91	2.97	1.90
Total	100.25	100.44	100.37	100.31	100.17	100.25
Humus nitrogen in soil, per cent	.073	.042	.067	.037	.064	.027

An examination of the physical constitution of the soil showed something more definite (Table II). Two samples were taken in each of several vineyards. One of these samples (marked "D") was taken from a spot where the vines were badly diseased, the other (marked "H") from a spot where the vines showed no symptoms of disease. In all cases sample D and its companion, sample H, were taken from spots as near together as possible and where there was no visible difference in color, texture, or situation.

TABLE II.—*Percentages of coarse material and of water in soil*[a]

W. VINEYARD (DIFFUSIVE TYPE OF ATTACK; NOT VERY SEVERE)

Date.	Depth.	Coarse material.			Moisture.		
		D	H	D−H	D_1	H_1	D_1+H_1
1912.	*Feet.*						
July	1	24.93	22.56	2.37	4.33	6.39	.....
Do	2	37.15	25.60	11.55	5.49	7.22	.....
Do	3	27.23	25.03	2.20	7.86	10.20	.....
Means	.....	29.77	24.39	5.38	5.89	7.93	74.2

[a] Determinations made by Mr. L. Bonnet.

TABLE II.—*Percentages of coarse material and of water in soil*—Continued

M. VINEYARD (DIFFUSIVE TYPE OF ATTACK; SEVERE. SOIL VERY LIGHT AND SANDY)

Date.	Depth.	Coarse material.			Moisture.		
		D	H	D−H	D_1	H_1	D_1+H_1
1912	*Feet.*						
July	1	59.60	43.09	16.51	2.86	0.88	.....
Do	2	67.38	39.21	28.17	4.84	1.68	.....
Do	A_3	72.98	44.86	28.12	4.77	1.71	.....
Do	4	75.18	40.89	34.29	4.38	1.98	.....
Do	1	66.00	51.30	14.70	3.88	2.19	.....
Do	2	72.13	48.93	23.20	4.66	3.87	.....
Do	B_3	63.81	52.45	11.36	3.83	3.28	.....
Do	4	62.40	52.55	9.85	3.89	3.77	.....
Do	1	52.04	45.63	6.41	3.79	3.34	.....
Do	2	56.25	51.06	5.16	4.85	4.43	.....
Do	C_3	60.70	49.81	10.89	4.76	2.60	.....
Do	4	57.65	49.36	8.29	4.50	2.36	.....
Means	.....	63.84	47.43	16.41	4.25	2.67	159

E. VINEYARD (INTENSIVE TYPE; VERY FEW VINES ATTACKED, BUT THESE SEVERELY. LOAM SOIL, RICH.)

1912							
July	1	18.05	16.86	1.19	1.64	1.33	.....
Do	2	21.02	17.68	3.34	4.42	3.26	.....
Do	3	19.80	18.58	1.22	7.00	5.49	.....
Do	4	18.75	20.00	−1.25	5.11	4.47	.....
Do	5	23.20	17.00	6.20	5.61	3.93	.....
Do	6	26.71	17.40	9.31	5.50	5.48	.....
Means	.....	21.26	17.92	3.34	4.88	3.99	122.3

S. VINEYARD (TYPE OF ATTACK INTERMEDIATE BETWEEN THE INTENSIVE AND THE DIFFUSIVE. MORE SEVERE THAN W., LESS THAN M.)

1912							
July	1	51.31	27.21	24.10	5.99	7.73	.....
Do	2	35.38	33.25	2.13	5.00	9.83	.....
Do	3	42.17	32.23	9.94	8.21	12.30	.....
Do	4	48.80	25.80	23.00	11.10	14.58	.....
Do	1	27.95	29.88	−1.93	5.15	6.17	.....
Do	2	39.02	31.88	7.14	6.70	6.75	.....
Do	3	45.55	33.25	12.30	8.43	11.81	.....
Do	4	41.90	27.53	14.37	11.44	11.82	.....
Do	1	35.74	22.78	12.96	4.34	6.08	.....
Do	2	37.48	26.81	10.67	5.65	6.85	.....
Do	3	39.94	31.45	8.49	9.16	8.91	.....
Do	4	35.45	30.45	5.06	11.10	12.07	.....
Means	.....	40.06	29.38	10.68	7.61	9.58	79.4
August	1	34.06	22.13	11.93	3.70	6.00	.....
Do	2	41.92	24.01	17.91	4.69	7.68	.....
Do	3	45.33	25.36	19.97	4.50	10.54	.....
Do	4	50.05	12.57	37.48	7.02	19.17	.....
Do	1	33.25	28.05	5.20	2.89	5.33	.....
Do	2	30.80	26.34	4.46	4.63	6.11	.....
Do	3	40.60	30.40	10.20	5.50	8.32	.....
Do	4	51.56	31.10	20.46	7.44	10.58	.....
Do	1	30.66	23.89	6.77	3.53	4.85	.....
Do	2	39.75	27.20	12.55	4.52	5.40	.....
Do	3	38.43	27.86	10.57	4.67	7.14	.....
Do	4	41.00	30.86	10.14	5.91	9.20	.....
Means	.....	39.78	25.81	13.97	4.91	8.36	58.7
September	1	34.48	25.35	9.13	3.38	6.05	.....
Do	2	38.33	24.38	13.95	3.66	6.62	.....
Do	3	39.00	25.00	14.00	4.26	10.85	.....
Do	4	44.28	22.10	22.18	5.48	13.95	.....
Do	1	33.26	26.13	7.13	3.17	5.12	.....
Do	2	34.93	27.90	7.03	3.69	5.09	.....
Do	3	39.86	29.20	10.66	4.54	6.53	.....
Do	4	35.70	30.95	4.75	5.70	8.95	.....
Do	1	33.06	22.28	10.78	2.38	3.62	.....
Do	2	33.41	22.10	11.31	2.66	4.19	.....
Do	3	33.48	24.95	13.53	2.95	5.38	.....
Do	4	36.05	30.98	5.07	3.60	8.30	.....
Means	.....	36.73	25.94	10.79	3.79	7.05	53.8

TABLE II.—*Percentages of coarse material and of water in soil*—Continued

S. VINEYARD (TYPE OF ATTACK INTERMEDIATE BETWEEN THE INTENSIVE AND THE DIFFUSIVE. MORE SEVERE THAN W., LESS THAN M.)—continued

Date.	Depth.	Coarse material.			Moisture.		
		D	H	D−H	D1	H1	D1+H1
1912	*Feet.*						
October	1	31.55	21.93	9.62	3.18	5.22	.....
Do	2	29.08	27.95	1.13	3.55	5.70	.....
Do	3	39.60	28.28	11.32	4.52	6.84	.....
Do	4	47.08	24.93	22.15	5.21	7.86	.....
Do	1	29.38	25.88	3.50	3.08	3.72	.....
Do	2	34.05	29.48	4.57	3.65	5.12	.....
Do	3	38.30	25.57	12.73	4.52	6.04	.....
Do	4	42.65	29.90	12.75	5.27	7.72	.....
Do	1	33.22	23.05	10.17	2.24	3.98	.....
Do	2	34.30	21.25	13.05	2.38	4.54	.....
Do	3	34.73	26.23	8.50	2.54	5.26	.....
Do	4	35.15	26.23	8.92	3.21	8.86	.....
Means	.....	35.76	25.89	9.87	3.61	5.91	61.1
November	1	29.56	22.75	6.81	3.56	6.71	.....
Do	2	37.58	25.88	11.70	3.49	7.43	.....
Do	3	39.23	25.13	14.10	4.15	9.13	.....
Do	4	40.05	19.28	20.77	4.61	12.52	.....
Do	1	20.50	24.00	−3.50	3.62	5.34	.....
Do	2	33.00	28.06	4.94	3.58	5.66	.....
Do	3	32.08	27.00	5.08	5.91	6.56	.....
Do	4	42.70	27.68	15.02	3.05	8.40	.....
Do	1	31.30	20.07	11.23	3.70	5.02	.....
Do	2	34.55	22.33	12.22	3.18	5.10	.....
Do	3	43.20	25.48	17.72	3.40	5.71	.....
Do	4	39.00	26.32	12.68	4.14	7.75	.....
Means	.....	35.23	24.50	10.73	4.03	7.11	56.7
1913							
March	1	28.98	20.90	8.08	3.24	5.91	.....
Do	2	33.28	22.27	11.01	3.24	7.57	.....
Do	3	35.00	20.64	14.36	3.63	8.32	.....
Do	4	39.38	20.22	19.16	4.56	10.61	.....
Do	1	26.26	24.22	2.04	3.80	4.65	.....
Do	2	28.63	26.46	2.17	4.10	5.60	.....
Do	3	30.10	25.27	4.83	4.44	6.63	.....
Do	4	40.50	26.12	14.38	5.27	8.32	.....
Do	1	29.30	22.15	7.15	3.71	5.17	.....
Do	2	31.30	22.38	8.92	3.81	5.42	.....
Do	3	33.43	24.38	9.05	4.05	5.97	.....
Do	4	33.70	24.20	8.50	4.46	7.45	.....
Means	.....	32.49	23.27	9.22	4.03	6.80	59.3
April	1	32.10	19.88	12.22	2.96	5.80	.....
Do	2	35.58	18.71	16.87	3.31	7.31	.....
Do	3	39.23	23.72	15.51	3.91	10.24	.....
Do	4	43.98	22.90	21.08	5.23	11.62	.....
Do	1	28.10	23.68	4.42	3.18	4.26	.....
Do	2	33.95	23.76	10.19	3.87	5.68	.....
Do	3	43.15	28.68	14.47	5.14	6.92	.....
Do	4	43.50	27.90	15.60	5.99	9.30	.....
Do	1	31.22	26.48	4.74	3.12	4.09	.....
Do	2	32.32	21.10	11.22	3.72	5.37	.....
Do	3	34.18	23.12	11.06	4.02	6.42	.....
Do	4	38.10	28.02	10.08	4.78	8.61	.....
Means	.....	36.28	24.00	12.28	4.10	7.14	57.4
May	1	28.55	21.75	6.80	3.00	6.61	.....
Do	2	35.48	24.07	11.41	3.93	8.26	.....
Do	3	33.86	22.06	11.80	4.84	10.09	.....
Do	4	38.85	21.65	17.20	6.63	13.35	.....
Do	1	29.86	23.48	6.38	3.87	4.96	.....
Do	2	27.97	26.56	1.41	4.13	6.35	.....
Do	3	34.41	30.75	3.66	5.08	7.62	.....
Do	4	40.75	33.13	7.62	7.55	9.74	.....
Do	1	27.13	23.90	3.23	3.74	5.53	.....
Do	2	32.72	24.85	7.87	3.83	6.22	.....
Do	3	43.38	25.88	17.50	5.02	6.91	.....
Do	4	44.38	27.20	17.18	6.41	9.46	.....
Means	.....	34.78	25.44	9.34	4.84	7.92	61.1

TABLE II.—*Percentages of coarse material and of water in soil*—Continued

S. VINEYARD (TYPE OF ATTACK INTERMEDIATE BETWEEN THE INTENSIVE AND THE DIFFUSIVE. MORE SEVERE THAN W., LESS THAN M.)—continued

Date.	Depth.	Coarse material.			Moisture.		
		D	H	D−H	D_1	H_1	D_1+H_1
1913	*Feet.*						
June	1	34.04	21.65	12.39	2.00	7.20	.....
Do	2	33.05	21.45	11.60	3.87	8.72	.....
Do	3	38.39	24.08	14.31	5.47	11.08	.....
Do	4	40.65	18.15	22.50	7.76	12.56	.....
Do	1	28.22	23.76	4.46	3.36	4.90	.....
Do	2	33.90	24.43	9.47	4.48	6.46	.....
Do	3	36.36	29.00	7.36	6.11	8.82	.....
Do	4	35.03	27.55	7.48	8.38	12.37	.....
Do	1	29.02	22.22	6.80	2.87	4.67	.....
Do	2	31.52	22.85	8.67	4.08	5.94	.....
Do	3	33.07	23.50	9.57	5.75	8.63	.....
Do	4	38.83	28.42	10.41	7.36	11.60	.....
Means	.....	34.34	23.92	10.42	5.12	8.58	59.7

In every case where the vines were diseased the soil shows more coarse material than the companion soil, where the vines were healthy. This is true in every vineyard examined and at all depths.[a] This uniformity indicates strongly a connection between the disease and the coarseness of the soil. That the coarse material is not alone responsible is shown by the fact that in the W. vineyard diseased vines were found where the proportion of coarse material was only 29.77 per cent, while in the M. vineyard healthy vines were growing in soil containing 47.43 per cent. It is to be noted, however, that the intensity of the disease is in proportion to the coarseness of the soil, both when we compare soils from different vineyards and where we compare soils from different parts of the same vineyard. The difference between the coarse material in the diseased parts of the vineyard and in the healthy parts (D − H, Table III) is roughly a measure of the intensity of the disease. Vineyard K. is a special case. The difference D − H is small, but the diseased vines were very badly affected. There were, however, but a few single vines or groups of two or three vines affected and all the rest appeared perfectly healthy.

TABLE III.—*Percentage of coarse material in diseased and in healthy portions of vineyard (summarized from Table II)*

Vineyard and character of attack.	Percentage of coarse material.		
	Diseased.	Healthy.	D−H.
M. Attack diffusive; severe	63.84	47.43	16.41
S. Attack intermediate; moderately severe	36.16	25.35	10.81
W. Attack diffusive; not severe	29.77	24.39	5.38
K. Attack intensive; very few vines affected, but these badly	21.26	17.92	3.34

[a] Of 129 pairs of samples examined, there were only 3 exceptions, and these apply to only a single foot and not to the whole soil depth.

The differences noted in the moisture contents of diseased and healthy spots were less concordant, as shown by Table IV.

TABLE IV.—*Moisture in diseased and in healthy portions of vineyard (from Table II)*

Vineyard and character of attack.	Percentage of moisture.		
	Diseased.	Healthy.	D÷H.
M. Attack diffusive; severe	4.25	2.67	159.0
S. Attack intermediate; moderately severe	4.62	7.61	60.7
W. Attack diffusive; not severe	5.89	7.93	74.2
K. Attack intensive; very few vines affected, but these badly	4.88	3.99	122.3

In the S. and W. vineyards the moisture contents were notably lower in the diseased spots than in the healthy, but in the other vineyards they were higher in about the same proportion. The most weight should be given to the results in vineyard S. on account of the large number (108) of determinations made. In every case in this vineyard, at all depths, the soil in diseased spots was drier than in healthy spots. The same is true of the W. vineyard. In the M. and the K. vineyards this condition is reversed. In M. the soil is excessively sandy, of the type known as "blow sand," and in K. the soil is the least sandy of all. Both these vineyards were irrigated, and the moisture conditions observed may not be typical of the whole year. The S. vineyard was not irrigated, and the observations cover the greater part of the year, from March to November. It seems fair to conclude, therefore, that a lack of moisture is a characteristic of affected soils.

ANNUAL AND SEASONAL VARIATIONS

The disease does not seem to be progressive—that is, it does not necessarily increase with succeeding years. A vineyard may be sufficiently affected one year to lose most of its crop and show practically no sign of the disease the next. The trouble returns, however, in subsequent years. In most affected vineyards it is noticeable every year, but with varying degrees of intensity. Very badly diseased vines seem never to recover, and they finally die. The annual variation in a moderately affected vineyard (St. vineyard) and in a badly affected vineyard (F. vineyard) are shown in Table V.

TABLE V.—*Percentage of annual variations of attack in the St. and F. vineyards*

Condition.	St. vineyard.		F. vineyard.			
	1905	1906	1910	1911	1912	1915
Vines dead or missing	0	0	9.7	[a] 2.5	2.5	3.2
Vines badly affected	0	10.5	56.8	29.9	33.3	40.1
Vines slightly affected	20.5	17.7	29.9	34.9	21.2	18.7
Vines healthy	79.5	71.8	12.6	38.7	43.0	38.1

[a] Missing vines of 1910 replaced with new plantings.

The St. vineyard consisted of old Tokay vines; the F. vineyard of young (5 years old in 1910) Zinfandel vines grafted on Rupestris St. George. In the F. vineyard, there was marked improvement in 1911, and a slight relapse during subsequent years.

The sequence of the symptoms during the year is peculiar. All vines, except those very badly affected, appear perfectly healthy during the early spring. It is only when the weather becomes warm, usually in May, June, or July, that the symptoms become marked. They increase until about the commencement of the vintage. At this time they commence to disappear and moderately affected vines may appear perfectly healthy in October and November. The healthy character of the first growth is indicated in Plate 89, *B*. The leaves on the lower part of the canes appear quite healthy. These are the first leaves formed in the spring. The disease became noticeable about the time of the appearance of the blossoms; and the result is shown in the small, abortive berries and the small leaves on the upper parts of the canes.

SYMPTOMS OF LITTLE-LEAF

LEAVES

Slightly affected leaves are of normal size, or nearly so, but show a tendency to curl up at the edges. They show bands or patches of light-colored parenchyma. The rest of the leaf appears somewhat darker than normal, but this may be the effect of contrast. Such leaves appear on very slightly affected vines or at the bases of canes more badly affected (Pl. 90, *B*).

More badly affected leaves are smaller and the bands of light colored parenchyma are larger and more numerous. These bands are not regular nor distinctly marked, but cause the general color of the foliage of a diseased vine to be lighter than that of a healthy one. Such leaves occur chiefly on the middle portions of the canes.

In very badly affected leaves these symptoms are intensified, and the light yellowish bands or spots dry, turn brown, and crack. When the canes are shaken, the leaves make a sound like dry leaves. They are so brittle that when folded they crack. The petioles are abnormally short and thick.

The microscopical examination of sections showed very little difference except a scarcity of chlorophyll grains in the diseased leaves. Though these feel thicker, micrometric observations showed that they were actually thinner than the corresponding healthy leaves. Ninety-three measurements of healthy and diseased leaves gave an average thickness of 194.3 μ for the former and 170.2 μ for the latter. These measurements were made on Feher Szagos and Zinfandel.

The shape of the leaf is not altered. A large number of measurements showed no change in the ratio of length to width.

The difference in size varied with the intensity of the disease, with the position of the leaf on the cane, and with the variety (Table VI).

TABLE VI.—*Comparison of size of healthy and diseased leaves of the vine*

Order of leaf on cane.	(Length of healthy leaf)÷(Length of diseased leaf).		
	Petite Sirah.	First Zinfandel.	Second Zinfandel.
1	.....	.....	.....
2	.....	1.11	1.56
3	0.81	.....	.....
4	.....	1.19	1.56
5	1.17	1.04	2.00
6	1.26	1.64	2.86
7	1.27	.....	.....
8	1.53	1.60	3.03
9	1.42	1.94	4.38
10	1.50	2.45	6.64
11	1.77	3.53	.....
12	.....	3.12	.....
Means	1.34	2.00	3.14

This table shows three degrees of intensity of attack. With the Petite Sirah there was little difference in size of leaves up to the fifth from the base of the cane. From this point the leaves on the diseased vines gradually became smaller, until at the eleventh node the diseased leaf was only 56 per cent as long as the corresponding healthy leaf. The first Zinfandel shows an increase of intensity. The lower leaves up to the fifth showed little difference, but the eleventh on the diseased vine was only 32 per cent as long as the healthy leaf. The second Zinfandel represents a very severe case. Even the lowest leaves were much smaller (64 per cent), and the tenth leaf of the diseased vine was only 15 per cent as long as the healthy leaf.

These measurements were made early in May and do not show the decrease of intensity shown later by the production of almost normal leaves.

CANES

As with the leaves, the symptoms on the shoots and canes vary with the intensity of the disease. The lower or earlier internodes may be nearly or quite normal, except in very severe cases. From the middle, and especially on the laterals, the internodes are shortened and flattened.

The shoots and canes are very erect. This, with the abnormal number of laterals, gives the vine a bushy appearance, completely changing its natural habit. In a vineyard of moderately affected vines the average length of the shoots of diseased vines was found to be 49 per cent of that of the shoots of healthy vines. This corresponds to the average difference between the lengths of the internodes, which was in the ratio of 100 to 50.

The flattening, or ratio between the longest and shortest diameters of the canes, is marked in some cases. Measurements made showed this ratio to average 1.22 in healthy vines and 1.38 in corresponding diseased vines.

ARMS

On the 2-year-old wood, from which the canes arise, the flattening is even more common and pronounced. The arms on badly diseased vines are shorter and less numerous than on healthy vines. This is due to the dying back of the second- and third-year-old wood and the removal of the arms in pruning. The water sprouts from old wood being usually larger and more vigorous than the fruit canes on younger wood, the pruner has a tendency to utilize them for spurs. This tends to keep the arms short.

A section through the 2-year-old part of an arm shows yellowish points usually confined to the 2-year-old wood. Sometimes these points are so numerous as to color the whole surface of the section and then a few points may occur on the 1-year-old wood. These yellow points occur also on apparently healthy arms, but rarely and never abundantly.

Sections made successively through older parts of the arm show more numerous and darker points. In wood 4 to 6 years old these points are nearly black. They are usually scattered through all annual growth rings except that of the current year.

The microscope reveals in these dark and yellowish spots the presence of thyloses and gum in the xylem tubes, parenchymatous cells and in a few xylem fibers adjoining them (Pl. 90, *A;* 91, *A, B*). The medullary rays close to affected tissues show dark cells filled with a granular and brown substance unstained by iodin.

TRUNK

Dark points like those in the arms are also found in the trunk. They can be followed almost to the base of the underground stem. At the head of the vine, where the arms start, occur the most abundant diseased areas. These gradually diminish in number as we approach the base of the underground stem. This progressive diminution is shown in Plate 91, *C–E*. This represents three sections through the trunk of a badly affected 3-year-old Petite Sirah vine. Figure *C* is a section at the branching point of the arms about 12 inches above the surface of the ground. The wood of the first two years is all of a uniform yellowish brown color. *D* is a section at the level of the ground. It shows 19 dark dots or small patches distributed through 2- and 3-year-old wood. *E* was made at 6 inches below the surface and shows only 9 of these dots. Only 3 dots were found in a section made at the base of the underground stem.

Microscopically, these diseased areas show the same gummy matters noted in other parts, but they are darker. When the tissues are stained

with carbol-fuchsin, the xylem fibers stain red and the xylem tubes orange, indicating an abnormal condition of the walls of the xylem tubes.

ROOTS

The diseased spots may be found in the large roots, which when split show dark longitudinal lines corresponding to these spots.

FRUIT

The effect on the crop has already been noted. On slightly affected vines the crop may be diminished considerably. Those at all badly affected have a tendency to fail to set their fruit, and much of that which does set fails to develop or mature.

A microscopical examination of the pedicels of diseased bunches shows that there is little activity in the cambium. A lack of thickening of the pericylic fibers and of the collenchyma is also noted which explains the fragility of the stems and failure of the berries to develop (Pl. 91, *F*, *G*).

INFECTIOUSNESS

There is little or nothing in the distribution of the disease to indicate an infectious nature. Some growers claim that the infected areas gradually enlarge, but they are probably misled by the annual variations already noted. It is also possible that vines, so slightly affected at first that the injury passes unnoticed, gradually weaken and show the effects more plainly later, thus giving the impression of a spread of the trouble.

In some vineyards the disease has existed in spots for years without spreading. Perfectly healthy vines may show symptoms of little-leaf in the nursery and yet develop into perfectly healthy vines when planted in another soil. On the other hand, vines which appear healthy in the nursery may show the disease the first year when planted in certain soils. In the latter case, however, the disease does not usually manifest itself until the third or fourth year.

ETIOLOGY

No connection has been found between the disease and any vegetable or animal organisms. Bacteria have been found in diseased tissues, but inoculations in various ways afforded no evidence that they have any connection with the disease.

Young vines showing the disease in affected nurseries have been planted in districts where little-leaf does not occur, and they have developed normally, showing no symptoms of the disease in the new location. On the other hand, healthy vines from an unaffected district may show the disease the first year after planting in an affected district. Both of these cases are exemplified by direct experiments made at Davis

and Fresno and by thousands of vines distributed by nurserymen. The evidence seems conclusive that the cause is local, whether it exists in soil, water, or weather conditions.

VARIATIONS IN SUSCEPTIBILITY

The disease has been noted on various species of Vitis, and none has proved immune. It has been found on the following phylloxera-resistant stocks: Riparia gloire de Montpellier, Rip. grand glabre, Rupestris St. George, Vitis aestivalis, V. Champini, V. Doaniana, Berl. × Rip. 157–11, Rip. × Rup. 101–14, 3306, 3309, Rip. × Cord. × Rup. 106–8. Sol. × Rip. 1615, 1616.

All varieties of *Vitis vinifera* are attacked, so far as noted; but a few appear to be peculiarly susceptible and a few others to have some considerable degree of resistance. The Mataro is so badly affected as to make a class by itself, although the Carignane is almost as bad.

Examples of partial resistance have been noted with Black Prince, Burger, Sultanina, Valdepeñas, Petite, and Alicante Bouschet. A vineyard of Mataro was found with all the vines nearly dead, except a few score scattering Black Prince vines which appeared perfectly healthy. Healthy Valdepeñas vines have been found in similar positions surrounded by badly affected vines of other varieties. Blocks of Sultanina, Burger, and the Bouschets, showing but negligible signs of disease, are growing adjacent to badly affected blocks of Carignane and other varieties. No badly affected vineyards or vines of these varieties have been found.

Badly affected vines in considerable quantities have been noted of Muscat, Tokay, Palomino, Feher Szagos, Zinfandel, Malaga, Green Hungarian, and Grenache.

Grafted vines seem to be as susceptible as vines on their own roots. The effect of grafting very susceptible or less susceptible varieties, and *vice versa*, has not been tried.

OTHER PLANTS AFFECTED

Several kinds of trees, differing very much botanically among themselves and from the vine, are affected by what seems to be the same trouble. This fortifies the idea that the disease is not infectious or parasitic. The location and distribution of the diseased trees are exactly parallel to those of the diseased vines, indicating a common cause. The Carolina poplar seems particularly susceptible, showing the small, pale leaves, abnormally numerous small branches, and generally stunted condition in a very marked degree. On affected poplars very remarkable examples of fasciation of the branches are common. Copious irrigation was noted in one case to increase the growth of these trees, but without apparently diminishing the other symptoms.

Apricot trees are also very badly affected with symptoms almost identical with those of the vine. Other trees, such as peach, walnut, almond, fig, and umbrella trees, show similar symptoms; but the resemblance to the trouble of the vine is less marked than in the cases of poplar and apricot. The symptoms have not been noted in any annual plant, even in the worst affected spots.

COMPARISON WITH OTHER VINE DISEASES

The symptoms distinguish little-leaf clearly from any other disease of the vine in California. To two European diseases, however, it shows a strong resemblance. These are "*mal nero*" and "*court noué*."

From the former it seems sufficiently distinguished by the different discoloration of the leaves. The "*mal nero*," moreover, is infectious and is not confined to special soils. The name "*court noué*," meaning short-noded, indicates one of the most characteristic symptoms of little-leaf. It appears to be applied, however, to several distinct diseases. One of these has been shown to be due to a minute arachnid attacking the leaves. Others have been traced to winter frosts and bacterial infections in the old wood. Others are unexplained and may be identical with our little-leaf.

CONTROL EXPERIMENTS

To seek to control a disease whose cause is unknown is to work in the dark. The need of a remedy, however, is so pressing that a considerable amount of work has been done in the hope of finding one.

INSECTICIDES AND FUNGICIDES

Affected vines were treated with various insecticides and fungicides on the supposition that the trouble might be due to some unperceived animal or vegetable parasite.

Vines were sprayed at the swelling of the buds, when the shoots were 6 inches long, and on the appearance of symptoms, with "Rex" and nicotine and with "Killthrips." No results were noticed.

Other vines were sprayed with Bordeaux mixture, iron sulphate, and dilute sulphuric acid in various combinations and at various times, but without any very noticeable results. Spraying in June with Bordeaux mixture produced some slight improvement for a few weeks, but later the treated vines were no better, if not worse, than the untreated. Other sprays had no noticeable effect.

Swabbing the vines when dormant with 5 and 10 per cent solutions of copper sulphate had no effect. Swabbing the pruning wounds with 0.5 and 0.1 per cent solutions of mercuric chlorid in winter resulted in the improvement of the vines early in the season, but later these vines looked worse than the untreated.

Injections of iron-sulphate solutions (1:500) and of copper-sulphate solutions (1:500 and 1:1,000) were made on a number of vines. The solutions were caused to enter the vine by means of a ½-inch hole bored halfway through the trunk near the surface of the ground. A metal tube was inserted into this hole and connected, by means of a rubber tube, to a funnel fastened to the head of the vine. The solution was poured into the funnel and was absorbed very readily. From 250 to 375 c. c. were used on each vine. These injections were made at various times from June 9 to September 10 in 1910. No results were obtained with the iron sulphate.

The copper sulphate produced some remarkable effects. One vine which received an injection of 375 c. c. of a 1 to 500 solution of copper sulphate on June 9 lost all its leaves in a few days, but a new growth started almost immediately, and by July 15 the vine was covered with a full supply of apparently healthy and normal foliage. A neighboring vine showing about the same degree of disease at the time of the injection was left untreated, and on July 15 had shown no signs of recovery (Pl. 92, *A*, *a*). All vines (11) which received injections of copper sulphate reacted in the same way. The first injury to the leaves and their subsequent recovery, however, were not so marked as with the first vine treated. The later the treatment, the smaller the effect. In all cases, however, the treated vines could readily be picked out by their more abundant, greener, and healthier looking foliage.

Examinations of the treated vines during the following year showed that the improvement had not been permanent. There was no apparent difference between the treated and the untreated vines when examined in 1911.

Similar injections were made with a larger number of vines during the following year in winter, spring, and in June. The winter injections apparently increased the disease. Those of spring and early summer had little effect, though some of those made in June improved the vines slightly. The vines treated this year were grafted, younger, less badly affected, and of a different variety.

CULTURAL MEASURES

The effect of fertilization was tried on about one-fifth of an acre in a patch of diseased vines. A complete fertilizer recommended by Prof. Burd was used at the rate of 400 pounds per acre. It had the following composition:

Superphosphate	P_2O_5 available	9.00	
Do	P_2O_5 insoluble	1.00	
			10.00
Sodium nitrate	N	1.50	
Ammonium sulphate	N	1.00	
Blood	N	2.50	
			5.00
Potassium sulphate	K_2O		4.00

Examinations made during the following seasons failed to show any difference in the amount of disease between the fertilized and the unfertilized portions of the patch.

It had been suggested by Mr. Frank Swett, of Martinez, and Prof. E. H. Twight that the trouble might be due to a deficiency of lime. Tests on nursery vines by Mr. Swett showed improvement in vines where lime had been used. Applications to old vines by Mr. Swett and others showed little or no effect. A test of the effect of gypsum was made by the California Agricultural Experiment Station on a small vineyard of 176 old Tokay vines, half of which was badly affected and half healthy or only

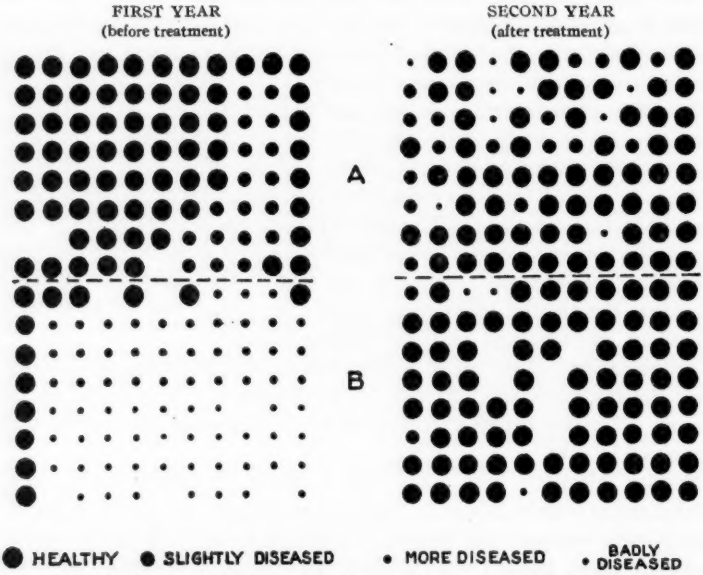


FIG. 2.—Graph showing the results of application of gypsum for the control of little-leaf. Gypsum was applied to the portion marked "B" during the winter of the first year. During the summer of the first year A was but slightly affected, B badly. During the summer of the second year A was a little worse, but B was almost free from disease symptoms.

slightly affected. The condition of the vines the year before the application was made and at a corresponding date of the year following the application is shown in figure 2 and Table VII.

The beneficial effect of the application of gypsum seems very apparent in this case. Block B, of which 83 per cent of the vines were diseased the year before the gypsum was applied, had only 5.8 per cent the following year—that is to say, the disease had almost disappeared. Block A, which had only 20.5 per cent of diseased vines the first year, had 29.6 per cent the second, showing that the improvement of block B was not due to the season. Unfortunately, this vineyard was close to a town, and was uprooted before further observations could be made.

TABLE VII.—*Results of application of gypsum to a vineyard for the control of little-leaf*

Block and condition.	First year.		Second year.	
	Vines.	Per cent.	Vines.	Per cent.
Block "A" (no treatment):				
Healthy	70	79.5	62	70.4
Slightly affected	18	20.5	16	18.2
More affected	.....	.....	.....	.....
Badly affected	.....	.....	10	11.4
Block "B" (1,000 pounds of gypsum per acre):				
Healthy	15	17.0	83	94.2
Slightly affected	.....	.....	2	2.3
More affected	33	37.5	.....	.....
Badly affected	40	45.5	3	3.5

Winter and early spring irrigation, so far as noted, seem to have little or no effect on the disease. The effect of summer irrigation has not been well tested.

Mr. A. Bonnet, of Montpellier, France, has suggested the plausible hypothesis that little-leaf is due to unfavorable changes of temperature, and specifically to a period of cold weather checking growth started by a previous period of warm weather. To test this hypothesis, measures were taken to delay the starting of the buds. This was accomplished by late pruning, whitewashing, and shading the vines. While a delay of nearly two weeks was obtained, no perceptible effect on the disease was noted.

SUMMARY

"Little-leaf" is a disease of the vine, vying in seriousness with oidium and phylloxera. It is confined to sandy soil in the great valley south of Sacramento County. It seems to be unknown elsewhere, unless a form of "*court noué*," noted in northern Mexico and southern Europe, are identical.

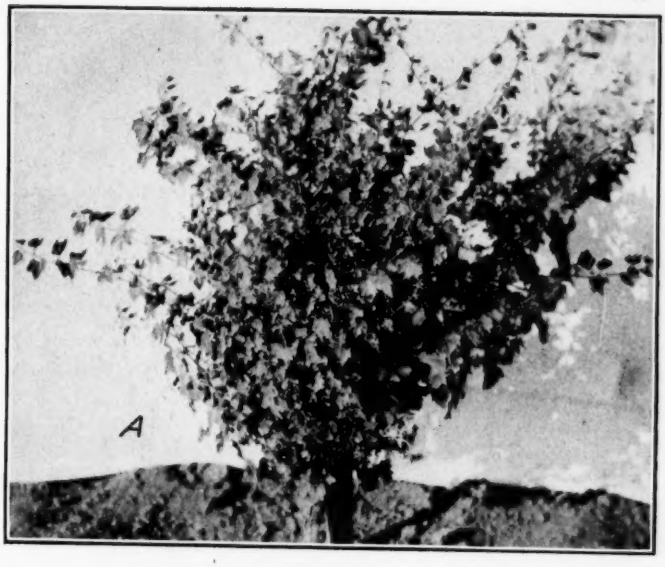
Affected vines show small, yellowish leaves, short-jointed canes, and in severe cases dead spots on the leaves and gummy secretions in the conducting tissues of the arms and trunks. In severe cases the vines die after a few years. Vines slightly affected set fruit imperfectly, and those badly affected bear little or nothing.

No parasite has been found connected with the disease, and the evidence seems to show that it is not infectious. Several species of trees are affected with similar symptoms when growing near or among affected vines, indicating that the disease is due to local conditions of soil, water, temperature, or other nonparasitic causes.

No effective method of treatment has been demonstrated, though the application of gypsum to the soil is promising.

PLATE 89

A.—A grapevine showing a severe infection of little-leaf.
B.—A moderately affected grapevine, showing healthy first leaves.


A

B

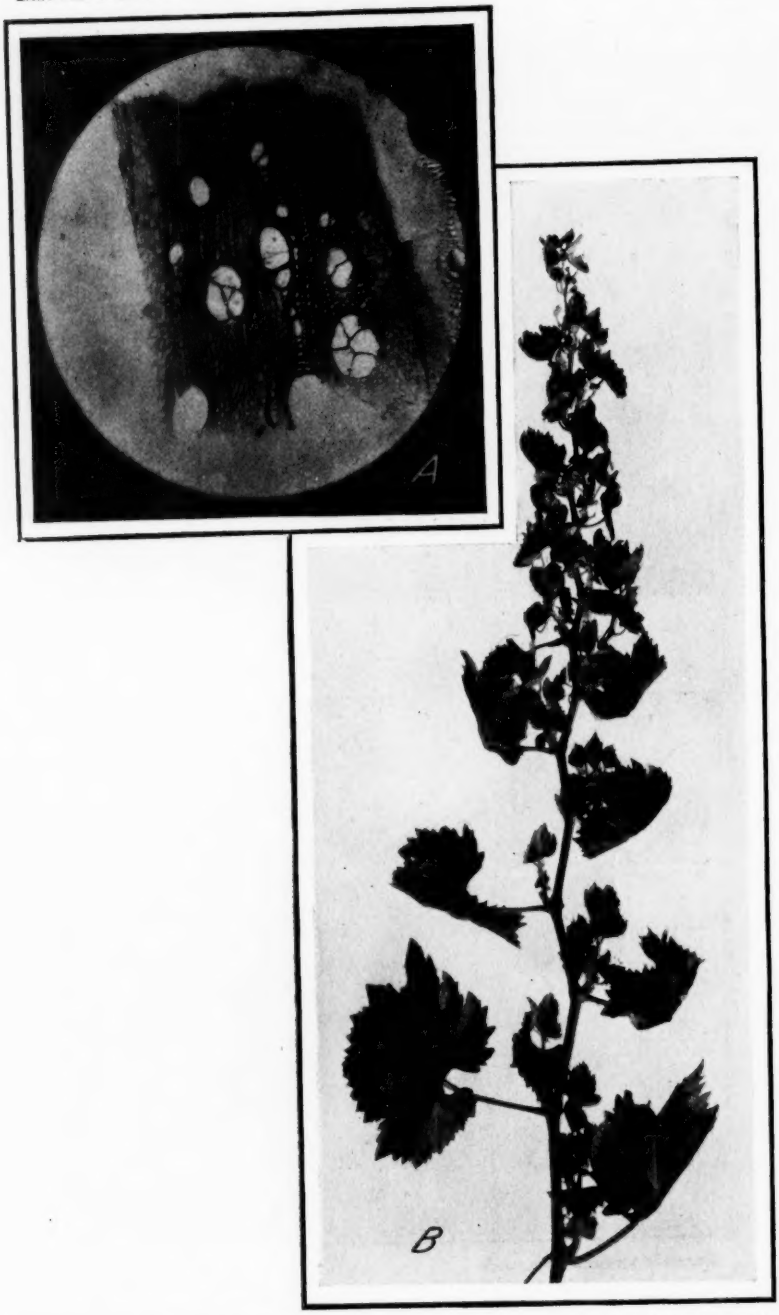
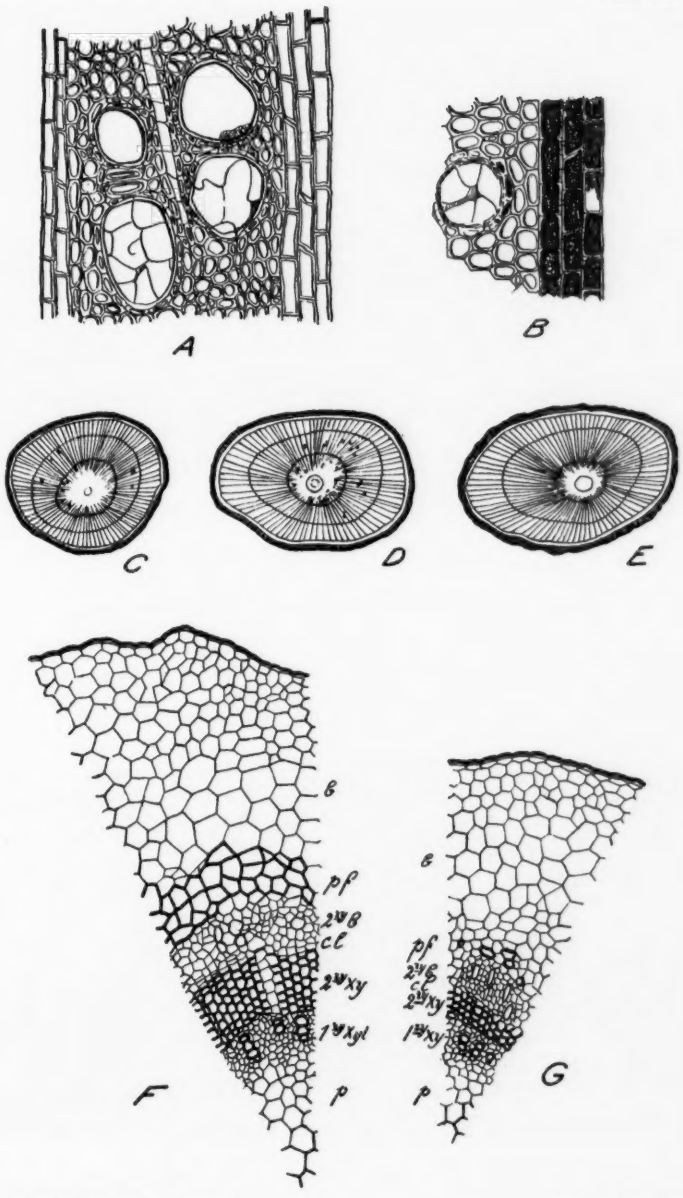

A
B

PLATE 90

A.—Tyloses in the wood of the grapevine.
B.—Grapevine shoots showing little leaves and laterals.

PLATE 91

A, B.—Drawings of tyloses in the wood of the grapevine.
C, D, E.—Cross sections of the trunks of the grapevine.
F, G.—Cross sections of healthy and diseased pedicels of the grapevine.


A
B
C
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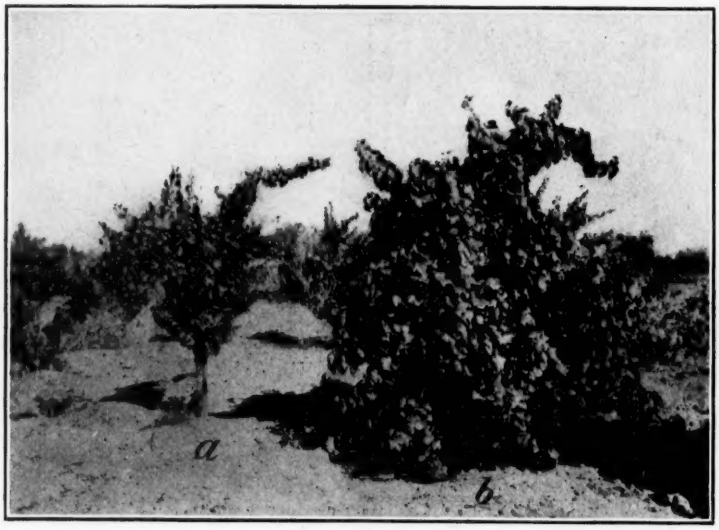

A

B

PLATE 92

A.—Grapevines showing the effect of treatment with an injection of copper sulphate: *a*, untreated; *b*, treated.

B.—Vineyard showing a severe case of the intensive form of little-leaf.